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(71) Applicant: MC MASTER UNIVERSITY [CA/CA]; 1200 Main Street West, Hamilton, Ontario L8N 3Z5 (CA).

(72) Inventors: GRAHAM, Frank, L.; 34 Amelia Street, Hamilton, Ontario L8P 2V4 (CA). BETT, Andrew; 1-3 Sterling Street, Hamilton, Ontario L8S 4H6 (CA). PREVEK, Ludvik; 944 LaSalle Park Road, Burlington, Ontario L7T 1M9 (CA). HADDARA, Wael; Unit H708, 644 Main Street West, Hamilton, Ontario L8S 1A1 (CA).

(74) Agents: MORROW, Joy, D. et al.; Smart & Biggar, 900 - 55 Metcalfe Street, P.O. Box 2999, Station D, Ottawa, Ontario K1P 5Y6 (CA).

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(57) Abstract

The invention comprises a series of adenovirus-based vectors having deletions in the E1 and/or E3 regions, and optionally insertions of pBR322 sequences, which can be used to deliver nucleic acid inserts into host cells, tissues or organisms that then can express the insert. The invention also comprises the use of these vectors in introducing genes into cells, in making vaccines and in gene therapy.

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ADENOVIRUS VECTORS FOR GENE THERAPY

This application claims priority from the following two applications: (1) U.S. Application Serial No. 08/080,727 filed June 24, 1993, entitled ADENOVIRUS VECTORS FOR GENE THERAPY; and (2) its continuation-in-part U.S. Application Serial No. 08/250,885, filed on May 31, 1994, entitled ADENOVIRUS VECTORS FOR GENE THERAPY. These applications are hereby incorporated by reference.

FIELD OF THE INVENTION

This invention relates to adenovirus (Ad) vectors that are useful for enhanced expression of selected nucleic acids in infected, transfected or transformed cells, especially eukaryotic mammalian cells. This invention also generally relates to the treatment of diseased states by using genetically engineered vectors that encode therapeutic substances useful as vaccines and for gene therapy.

BACKGROUND

Adenoviruses (Ads) are a relatively well characterized, homogeneous group of viruses. Roughly 100 different adenoviruses, including nearly 50 serotypes isolated from humans, have been identified to date.

Most common serotypes of Ads are nonpathogenic, physically and genetically stable, can be grown to very high titres (concentrated stocks with 10^{11}-10^{12} PFU/ml of

infectious virus are easy to obtain) and easily purified by isopycnic centrifugation in CsCl gradients. The Ad genome is readily manipulated by recombinant DNA techniques, and the proteins encoded by foreign DNA inserts that are expressed in mammalian cells will usually be appropriately glycosylated or phosphorylated, unlike recombinant proteins expressed in bacteria, yeast, and some insect cells. Although human Ads replicate most efficiently in human cells of epithelial origin, these viruses infect almost any mammalian cell and express at least some viral genes. Unlike retroviruses, Ads will infect, and are expressed in, nonreplicating cells. Thus, Ad-based vectors may be useful for gene delivery, expression, and gene therapy.

Ad vectors have been constructed by ligation or recombination of viral DNA with subgenomic viral sequences contained in bacterial plasmids. Berkner, K.L. and Sharp, P.A., 1983, *Nucleic Acids Res.* 11: 6003-6020; Haj-Ahmad, Y. and Graham, F.L., 1986, *J. Virol.* 57: 267-274; Stow, N.D., 1981, *J. Virol.* 37: 171-180. This approach has several drawbacks, which include the time and technical difficultly required to produce viral DNA, the background of infectious parental virus which makes screening more labor intensive and, in the case of direct ligation, the limited availability of useful restriction sites due to the relatively large size of the adenovirus genome.

Another strategy has been to recombine two plasmids which together contain sequences comprising the entire Ad genome. A number of conditionally defective plasmid systems have been developed making the construction of vectors simpler and reducing the number of subsequent analyses required to identify recombinant viruses. McGrory, W.J., Bautista, D.S. and Graham, F.L., 1988, *Virol.* 163: 614-617; Ghosh-Choudhury, G., Haj-Ahmad, Y., Brinkley, P., Rudy, J. and Graham, F.L., 1986, *Gene* 50: 161-171; Mittal, S.K., McDermott, M.R. Johnson,

D.C., Prevec, L. and Graham, F.L., 1993, *Virus Res.* 28: 67-90.

The representative Adenovirus 5 ("Ad5") genome used in embodiments of the present invention is a 36kb linear duplex. Its sequence has been published. (Chroboczek, J., Bieber, F., and Jacrot, B., 1992, The Sequence of the Genome of Adenovirus Type 5 and Its Comparison with the Genome of Adenovirus Type 2, *Virol.* 186: 280-285; hereby incorporated by reference). The Ad5 genome contains a 100-150 base pair (bp) inverted terminal repeat ("ITR") at each end of the linearized genome. A terminal protein ("TP") of 55,000 daltons is covalently linked to the 5' end of each strand. Both the TP and the ITRs are thought to play a role in viral DNA replication. McGrory, W. J. *et al.*, 1988, *Virol.* 163: 614-617 and Ghosh-Choudhury, G. *et al.*, 1986, *Gene* 50: 161-171.) Ad5 has infected each human cell line tested, although some cells, such as lymphocytes, are relatively nonpermissive.

Four noncontiguous regions of the Ad5 genome are transcribed early in infection, prior to DNA replication. These regions are early region 1 (E1) (about 1.3-11.2 mu of or about position 198-4025 bp of a standardized genome, inclusive of the E1A enhancer region; Sussenbach, J. S., 1984, *in* Ginsburg (Ed.), THE ADENOVIRUSES, Plenum Press, pp. 35-124) which is further divided into subregions E1A and E1B; early region 2 (E2), which encodes the DNA replicative functions of the virus; early region 3 (E3) (about 75.9-86.0 mU, or about 27,275-30,904 bp; Cladaras, C. and Wold, W.S.M., 1985, *Virol.* 140: 28-43; and early region 4 (E4). E1A is involved in turning on the other early regions and in regulating a number of host cell functions. E1B and E4 are primarily involved in shutting off the host cell's protein synthesis. E3 regulates the host cell's immune response to virus infection. Some of these early genes function to "turn on" later-expressed genes that are needed to

replicate the genome and form viable viral particles.

The Ad virion has the ability to package up to 105-106% of the wild type genome length. Bett, A.J., Prevec, L., and Graham, F.L., 1993, Packaging Capacity and Stability of Human Adenovirus Type 5 Vectors, *J. Virol.* 67: 5911-5921. Larger genomes (e.g., 108% of the wild type in size), result in instability of the virus and poor growth rates. *Id.* This packaging ability allows the insertion of only approximately 1.8-2.0 kb of excess DNA into the Ad genome.

To package larger inserts, it is necessary to first delete portions of the viral genome. Parts of region E1 can be deleted, and the resulting viruses can be propagated in human 293 cells. (293 cells contain and express E1, complementing viral mutants that are defective in E1.) Foreign nucleic acids can be inserted in place of E1, in Ad5 genomes that contain E1 deletions of up to 2.9 kb, to yield conditional helper-independent vectors with a capacity for inserts of 4.7-4.9 kb.

Viruses with a region E3 deletion can also replicate in cultured human cells such as HeLa or KB and infect and be expressed in animals including humans. A deletion of a 3.0 kb E3 sequence has been reported, without a concomitant insertion. Ranheim, T.S., Shisler, J., Horton, T.M., Wold, L.J. Gooding, L.R., and Wold, W.S.M., 1993, *J. Virol.* 67: 2159-2167.

Among the methods developed to date there is no simple procedure for generating vectors that utilize both E1 and E3 deletions. In addition, the vectors that do utilize either E1 or E3 deletions can accomodate only relatively small inserts. To simplify the production and use of Ad vectors that can tolerate larger fragments, we have developed a new methodology based on a series of bacterial plasmids that contain most of an Ad viral genome.

SUMMARY OF THE INVENTION

It is a goal of this invention to provide simple, flexible, efficient, high capacity Ad 5 cloning and expression vectors. Accordingly, a new vector system has been developed which comprises expanded deletions in both E1 and E3 and further combines them in a single vector system that can tolerate inserts of up to 8000 bp of inserts, enough to accommodate the majority of protein coding genes along with control elements to regulate expression. The invention provides the option of cloning foreign nucleic acids into either or both of the E1 or E3 regions and promises to be the most versatile and easy to use technology yet developed. In addition, a modification of the system permits construction of viruses carrying a wild type E3 region, and insertions, substitutions, or mutations in the E1 region.

One embodiment of the present invention provides a bacterial plasmid comprising a circularized modified human adenovirus type 5 (Ad5) genome. The nucleotide sequence of the plasmid has a deletion within early region 3 (E3) of said Ad5 genome, and a segment of bacterially replicable pBR322 plasmid encoding ampicillin resistance substituted for a sequence of early region 1A (E1A) that corresponds, in whole or in part, to the packaging signal.

Another embodiment provides a bacterial plasmid comprising approximately 340 base pairs from the left end of the adenovirus type 5 genome, the left end inverted terminal repeat sequences of said genome and the packaging signal sequences thereof, said plasmid comprising also a eukaryotic gene sequence of up to about 8 kilobases foreign to said plasmid and to said viral genome. The adenovirus sequence from approximately nucleotide position 3540 thereof to approximately position 5790 thereof is present on the right side of said foreign sequence.

Other embodiments of the present invention include adenovirus genome constructs containing E1 deletions and foreign inserts of eukaryotic origin, using any combination of size of E1 deletion and/or of size of foreign insert that can be accommodated in the plasmid and still remain operable. Because of the large capacity of the vectors provided herein, multiple inserts of foreign genes can be placed in the E1 cloning site. For example, two or more genes encoding different antigens, or genes encoding useful proteins, can be combined with genes encoding chemically selectable markers.

One specific embodiment of the invention, the plasmid pBHG10, may be used to insert foreign genes into either the E3 or E1 region of the Ad5 genome. Genes inserted into E3 can be combined with a variety of mutations, deletions, or insertions in E1 by appropriate choice of the cotransfected plasmid containing left end (E1) sequences.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagrammatic representation of the structure and construction of the vector pBHG10.

Fig. 2 is a diagrammatic representation of the structure and construction of the vector pBHG3.

Fig. 3 is a diagrammatic representation of rescue using pBHG vectors.

Fig. 4 is a diagrammatic representation of the structure and construction of a 3.2 kb E1 deletion, and two examples (pΔE1sp1A and pΔE1sp1B) of plasmids that contain said deletion.

Fig. 5 illustrates the different levels of protein IX synthesized using plasmids having different E1 deletions with or without a reintroduced Ssp1 site.

Fig. 6 illustrates heat stability of viruses with the 3.2 kb E1 deletion with or without a reintroduced Ssp1 site.

Fig. 7 illustrates the construction and rescue of a 7.8 kb insert using pBGH10.

Fig. 8 depicts the strategy for the construction of a double recombinant containing lacZ in the E3 deletion and firefly luciferase in the E1 deletion.

Fig. 9 is a diagrammatic representation of the plasmids pABS.6, pABS.7, and pABS.9.

Fig. 10 is a diagrammatic representation of the shuttle plasmids pHCMVsp1A, pHCMVsp1B, pHCMVsp1C, and pHCMVsp1D.

DETAILED DESCRIPTION OF THE INVENTION

The recombinant Ad vectors provided herein are significantly different from previously reported constructs partly because they contain the largest possible deletion of E1 sequences (within 30-40 bp) that can be made while still allowing the generation of viable viral recombinants. Surprisingly, the different genetic elements described herein, when combined, produced a stable construct useful in introducing and expressing foreign nucleic acids in host cells.

At the onset of these experiments, it was unknown how large a deletion could be, or where it could be placed, without affecting the growth, production and infectivity of packaged virions. For viral viability and maximum packaging capacity, deletions in the E1 region preferably should not affect the left inverted terminal repeat (ITR; 1-103 bp) or packaging signals (194-358 bp). Hearing, P. and Shenk, T., 1983, *Cell*, 33: 695-703; Grable, M. and Hearing, P., 1992, *J. Virol.* 64: 2047-2056. In addition, because the only currently available E1 complementing cell line (293 cells) does not express protein IX, deletions should not extend into the coding sequences for this polypeptide. (Although viral deletion mutants lacking the protein IX gene have been isolated, it appears that the protein is essential for packaging of full length genomes into functional virus.)

In the pBHG plasmid embodiments of the invention, the pBR322 sequences substitute for Ad5 sequences from position 188 to 1339, which include the packaging signal, E1A enhancer, promoter and most of the E1A protein coding sequences. The pBR322 insert not only contains an ampicillin resistance, but allows allows the pBHG family of vectors to be replicated in cells wherein pBR322 may be replicated.

Some embodiments of the invention herein contain a deletion of the E1 region between an Ssp I site

at 339 bp and an Afl site at 3533 bp. Since the Ssp1 site may be essential for protein IX expression, it was reintroduced as a synthetic oligonucleotide which positioned the SspI site closer to the protein IX TATA box than is the case in the wild type (wt) protein IX gene.

Definitions

All technical and scientific terms used herein, unless otherwise defined, are generally intended to have the same meaning as commonly understood by one of ordinary skill in the art. A number of the terms used herein are not intended to be limiting, even though common usage might suggest otherwise. For example, the term "expression of" or "expressing" a foreign nucleic acid, gene or cDNA is used hereinafter to encompass the replication of a nucleic acid, the transcription of DNA and/or the translation of RNA into protein, in cells or in cell-free systems such as wheat germ or rabbit reticulocytes; and "nucleic acid" is used interchangeably with gene, cDNAs, RNA, or other oligonucleotides that encode gene products. The term "foreign" indicates that the nucleic acid is not found in nature identically associated with the same vector or host cell, but rather that the precise association between said nucleic acid and the vector or host cell is created by genetic engineering. The terms "recombinant" and "recombination" generally refer to rearrangements of genetic material that are contemplated by the inventors, and that are the result of experimental manipulation.

"Vector" denotes a genetically engineered nucleic acid construct capable of being modified by genetic recombinant techniques to incorporate any desired foreign nucleic acid sequence, which may be used as a means to introduce said sequence in a host cell, replicate it, clone it, and/or express said nucleic acid sequence,

wherein said vector comprises all the necessary sequence information to enable the vector to be replicated in host cells, and/or to enable the nucleic acid sequence to be expressed, and/or to enable recombination to take place, and/or to enable the vector to be packaged in viral particles. This recitation of the properties of a vector is not meant to be exhaustive.

Vectors, optionally containing a foreign nucleic acid, may be "introduced" into a host cell, tissue or organism in accordance with known techniques, such as transformation, transfection using calcium phosphate-precipitated DNA, electroporation, gene guns, transfection with a recombinant virus or phagemid, infection with an infective viral particle, injection into tissues or microinjection of the DNA into cells or the like. Both prokaryotic and eukaryotic hosts may be employed, which may include bacteria, yeast, plants and animals, including human cells.

A vector "supports the expression of coding sequences contained by the vector" when it serves as a vehicle for the introduction of a gene into a host cell, when sequences present in the vector enable the vector and the coding regions that it contains to be replicated and to be maintained in a cell without being degraded, and when sequences present in the vector enable the coding sequences to be transcribed, recombined, or integrated into the host cell genome.

Once a given structural gene, cDNA or open reading frame has been introduced into the appropriate host, the host may be grown to express said structural gene, cDNA or open reading frame. Where the exogenous nucleic acid is to be expressed in a host which does not recognize the nucleic acid's naturally occurring transcriptional and translational regulatory regions, a variety of transcriptional regulatory regions may be inserted upstream or downstream from the coding region, some of which are externally inducible. Illustrative

transcriptional regulatory regions or promoters for use in bacteria include the β-gal promoter, lambda left and right promoters, trp and lac promoters, trp-lac fusion promoter, and also the bacteriophage lambda P_L promoter together with the bacteriophage lambda O_L operator and the CI857 temperature-sensitive repressor, for example, to provide for temperature sensitive expression of a structural gene. Regulation of the promoter is achieved through interaction between the repressor and the operator. For use in yeast, illustrative transcriptional regulatory regions or promoters include glycolytic enzyme promoters, such as ADH-I and -II promoters, GPK promoter, and PGI promoter, TRP promoter, etc.; for use in mammalian cells, transcriptional control elements include SV40 early and late promoters, adenovirus major late promoters, etc. Other regulatory sequences useful in eucaryotic cells can include, for example, the cytomegalovirus enhancer sequence, which can be fused to a promoter sequence such as the SV40 promoter to form a chimeric promoter, or can be inserted elsewhere in the expression vehicle, preferably in close proximity to the promoter sequence. Where the promoter is inducible, permissive conditions may be employed (for example, temperature change, exhaustion, or excess of a metabolic product or nutrient, or the like).

When desired, expression of structural genes can be amplified by, for example, ligating in tandem a nucleic acid for a dominant amplifiable genetic marker 5' or 3' to the structural gene and growing the host cells under selective conditions. An example of an amplifiable nucleic acid is the gene for dihydrofolate reductase, expression of which may be increased in cells rendered resistant to methotrexate, a folate antagonist.

The expression vehicles used or provided herein may be included within a replication system for episomal maintenance in an appropriate cellular host, they may be provided without a replication system, or they may become

integrated into the host genome.

While a wide variety of host cells are contemplated, certain embodiments require that the host cell express E1 sequences that are missing from or inactivated in the vector. While the human 293 cell line is the preferred host cell, the invention also contemplates other cell lines capable of complementing the vector having an E1 deletion. "Complementing" or "complemented by" denotes that the host cell line encodes and/or expresses functions that are necessary for generating viable viral particles that are missing from or have been inactivated in the vector.

It is important to recognize that the present invention is not limited to the use of such cells as are used herein. Cells from different species (human, mouse, etc.) or different tissues (breast epithelium, colon, neuronal tissue, lymphocytes, etc.) may also be used.

"Modification" of a nucleic acid includes all molecular alterations of a nucleic acid sequence that change its capacity to perform a stated function, specifically including deletions, insertions, chemical modifications, and the like. Insertions and deletions may be made in a number of ways known to those skilled in the art, including enzymatically cutting the full length sequence followed by modification and ligation of defined fragments, or by site-directed mutagenesis, especially by loop-out mutagenesis of the kind described by Kramer *et al.*, 1984, *Nucl. Acids Res.* 12: 9441-9456.

"Fragment" refers to an isolated nucleic acid derived from a reference sequence by excising or deleting one or more nucleotides at any position of the reference sequence using known recombinant techniques, or by inserting a predetermined nucleotide or sequence of nucleotides at any predetermined position within the reference sequence using known recombinant techniques, or by substituting a predetermined nucleotide or sequence of nucleotides for a predetermined nucleotide or sequence of

nucleotides within the reference sequence using known recombinant techniques. It is not intended that the invention be limited to the use of nucleic acid sequences from any particular species or genus, but that this invention can be carried out using nucleic acids from a variety of sources. It is contemplated that any nucleic acid from any source may be inserted into the vector, with or without control elements.

"Gene therapy" comprises the correction of genetic defects as well as the delivery and expression of selected nucleic acids in a short term treatment of a disease or pathological condition.

Reference to particular buffers, media, reagents, cells, culture conditions and the like, or to

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In einer aus dem gleichen Gewebe erhaltenen Zellkernfraktion erkennt anti-"B" ein hochmolekulares Protein von ca. 70 k (**Fig. 2**, Bahn f). Zum Vergleich wird die Reaktion mit anti-MDGI-Antikörpern entweder als IgG-Fraktion (Bahn a) oder nach Affinitätsreinigung (Bahn c) bzw. mit gegen Peptid "A" gewonnenen Antikörpern (Bahn e) gezeigt. Als Spezifitätskontrolle dient erneut die Aufhebung der Bandenfärbung im Falle der Inkubation mit den entsprechenden Antikörpern in Gegenwart von authentischem MDGI (Bahnen b und d).

Da "B" ein biologisch mit MDGI gleichsinnig wirksames Peptid ist, deutet die Tatsache der Erkennung des 70 k — Kernproteins durch anti-"B" darauf hin, daß eine funktionelle Beziehung zwischen diesem 70 k-Protein und MDGI bzw. "B" besteht. Es ist zu vermuten, daß das 70 k-Kernprotein ähnliche biologische Wirkungen hat wie MDGI.

Fig. 2

Aus dem Homogenat von laktierender Rindermilchdrüse wurde eine Zellkernfraktion isoliert. Die entsprechenden Proteine wurden mittels Polyakrylamidgelelektrophorese in Gegenwart von SDS aufgetrennt (12,5% Gel) und auf Nitrozellulose transferiert. Der Immunnachweis erfolgte wie in **Fig. 1** beschrieben. Bahn a, b: Inkubation mit anti-MDGI-IgG

Bahn c, c: Inkubation mit affinitätsgereinigtem anti-MDGI-Antikörper
Bahn a, c: in Abwesenheit, Bahn b, d in Anwesenheit eines Überschusses von MDGI
Bahn e: Inkubation mit anti-"A"-Antikörpern
Bahn f: Inkubation mit anti-"B"-Antikörpern

Patentansprüche

1. Antikörper gegen biologisch aktive Peptide, die Teilsequenzen des Proliferationshemmstoffes MDGI (Mammary-Derived Growth Inhibitor) sind.
2. Antikörper gegen das Peptid EFDETTADDR.
3. Antikörper gegen das Peptid TAVCTRVYEKQ.
4. Antikörper gegen das Peptid TRVCTRVYEKQ.
5. Antikörper gegen das Peptid TAVSTRVYEKQ.
6. Verfahren zur Gewinnung von Antikörpern gegen biologisch aktive Peptide, dadurch gekennzeichnet, daß man Peptide, die identische oder modifizierte Teilsequenzen des Proliferationshemmstoffes MDGI (Mammary-Derived Growth Inhibitor) sind, durch ein Vernetzungsreagenz an ein Trägermolekül koppelt, Versuchstiere immunisiert, Antiseren gewinnt, die IgG-Fraktion isoliert und die spezifischen Antikörper mittels Affinitätschromatographie an trägergekoppeltem MDGI isoliert.
7. Verfahren nach Anspruch 6, dadurch gekennzeichnet, daß man als Peptide die einsetzt, die proliferationshemmende Wirkungen auf in vitro kultivierte Zellen und andere biologische Effekte, wie Desensibilisierung von Herzmuskelzellen gegenüber der Wirkung von beta-adrenergen Agonisten zeigen.
8. Verfahren nach Ansprüchen 6 und 7, dadurch gekennzeichnet, daß man als Peptid den identischen Sequenzabschnitt 121—131 von MDGI einsetzt.
9. Verfahren nach Ansprüchen 6 und 7, dadurch gekennzeichnet, daß man als Peptid einen modifizierten Sequenzabschnitt 121—131 von MDGI — TAVSTRVYEKQ einsetzt.
10. Peptid A, Sequenzabschnitt 69—78 von MDGI, EFDETTADDR.
11. Peptid B, Sequenzabschnitt 121—131 von MDGI, TAVCTRVYEKQ.
12. Peptid C, modifizierter Sequenzabschnitt 121—131 von MDGI, TRVCTRVYEKQ.
13. Peptid D, modifizierter Sequenzabschnitt 121—131 von MDGI, TAVSTRVYEKQ.

Hierzu 1 Seite(n) Zeichnungen

Abb. 4: Homologievergleich der zur Zeit bekannten FABPs der Spezies Maus bzw. Ratte. Im Anschluß an die Sequenzen wurde die Homologie im Vergleich zum CXBP in Klammern angegeben.

```
mCXBP      MVD--AFCATWKLTDSQNFDEYMKALGVGFATRQVGNVT--KPTVIISQEGGKVV      51
mH-FABP    .A.--..VG....V..K...D...S..........SM.--...T..EKN.DTIT      51
mA-FABP    .C.--..VG....VS.E...D...EV.......K.AGMA--..NM...VN.DL.T     51
mM-FABP    .SN--K.LG....VS.EH..D........L.N.KL..LA--.......KK.DYIT     51
mK-FABP    .ASLKDLEGK.R.ME.HG.E....E....L.L.KMAAMA--..DC..TCD.NNIT     53
rCRBP      .P--VD.NGY..MLSNE..E..LR..D.NV.L.KIA.LL--..DKE.V.D.DHMI     51
rCRBP2     .T--KDQNG..EMESNE..EG.....DID....KIAVRL--TQ.K..V.D.DNFK     51
mCRABP1    .PN---.AG...MRS.E....LL.....NAML.K.AVAAAS..H.E.R.D.DQFY     52
mCRABP2    .PN---.SGN..IIR.E..E.ML.....NMMM.KIAVAAAS..A.E.K..NDTFY     52
rI-FABP    .A----.DG...VYRNE.YEKF.EKM.INVVK.KL.AHDNLK--LT.T...N.FT     49
rL-FABP    M----N.SGKYQVQSQE..EPF...M.--LPEDLIQKGKDI.GVSE.VH..K..K     49
                        .  .  .   .  ..  .  ..       .     .    *  ..

mCXBP      IRTQCTFKNTEINFQLGEEFEE--TSIDDRNCKSVVRLDGDKLIHVQKW----DG     100
mH-FABP    .K..S..........I..D.--VTA...KV..L.T...G.......----..       100
mA-FABP    ..SES.......S.K..V..D.--ITA...KV..IIT...GA.VQ....----..     100
mM-FABP    ...ESA......S.K..Q..D.--.TA.N.KA..I.T.ERGS.KQ....----..     100
mK-FABP    VK.ES.V.T.VFSCN...K.D.--.TA.G.KTET.CTFQDGA.VQH.Q.-----..    102
rCRBP      ...LS..R.YIMD..V.K....DL.G....K.MTT.SW.....QC...G----EK     102
rCRBP2     TK.NS..R.YDLD.TV.V..D.HTKGL.G..V.TL.TWE.NT.VC.Q.G----EK     102
mCRABP1    .K.ST.VRT.....KV..G...E--TV.G.K.R.LPTWENENK..CTQTLLEG..     105
mCRABP2    .K.ST.VRT.....KI......Q--TV.G.P...L.KWESGNKMVCEQRLLKGE.     105
rI-FABP    VKESSN.R.IDVV.E..VD.AYSL--A.GTELTGTLTME.NK.VGKF.RV--DN.     100
rL-FABP    LTITYGS.VIHNE.T....C.--LETMTGEKV.A..KME..NKMVTT---FKGIK      99
                     . .      .*   .      ..    .    . .

mCXBP      KETNCTREI-KDGKMVVTLTFGDIVAVRCYEK-A      132      (100%)
mH-FABP    Q..TL...L-V...LIL...H.SV.ST.T...E.      133      (88%)
mA-FABP    .S.TIK.KR-DGD.L..ECVMKGVTST.V..R-.      132      (70%)
mM-FABP    ...AIR.TL-L..R...ECIMKGV.CT.I...-V      132      (72%)
mK-FABP    ..STI..KL-.....I.ECVMNNATCT.V...VQ      135      (51%)
rCRBP      EGRGW.QW.-EGDELHLEMRAEGVTCKQVFK.VH      135      (45%)
RCRBP2     ENRGWKQWV-EGD.LYLE..C..Q.CRQVFK.-K      134      (40%)
mCRABP1    FK.YW...LAN.-ELIL.FGAD.V.CT.I.VR-E      137      (42%)
mCRABP2    PK.SWS..LTN..ELIL.M.AD.V.CT.V.VR-E      138      (39%)
rI-FABP    ..LIAV...S-GNELIQ.Y.YEGVE.K.IFK.-E      132      (32%)
rL-FABP    SV.EFN-----GDTITN.M.L....YK.VSKRI-      127      (31%)
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Patentansprüche

1. Sequenzen des Maus-Cellular X Binding Protein (CXBP) kodierenden Gens Cxbp und homologer Gene anderer Säugerspezies, die durch Kreuzhybridisierung mit der dargestellten Gensequenz isoliert werden können.